
بسم الله الرحمن الرحيم

Biological Application of UV-Vis Spectrophotometry

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Spectroscopic Techniques and Chemistry they Probe		
UV-vis	UV-vis region	bonding electrons
Atomic Absorption	UV-vis region	atomic transitions (val. e-)
FT-IR	IR/Microwave	vibrations, rotations
Raman	IR/UV	vibrations
FT-NMR	Radio waves	nuclear spin states
X-Ray Spectroscopy	X-rays	inner electrons, elemental
X-ray Crystallography	X-rays	3-D structure

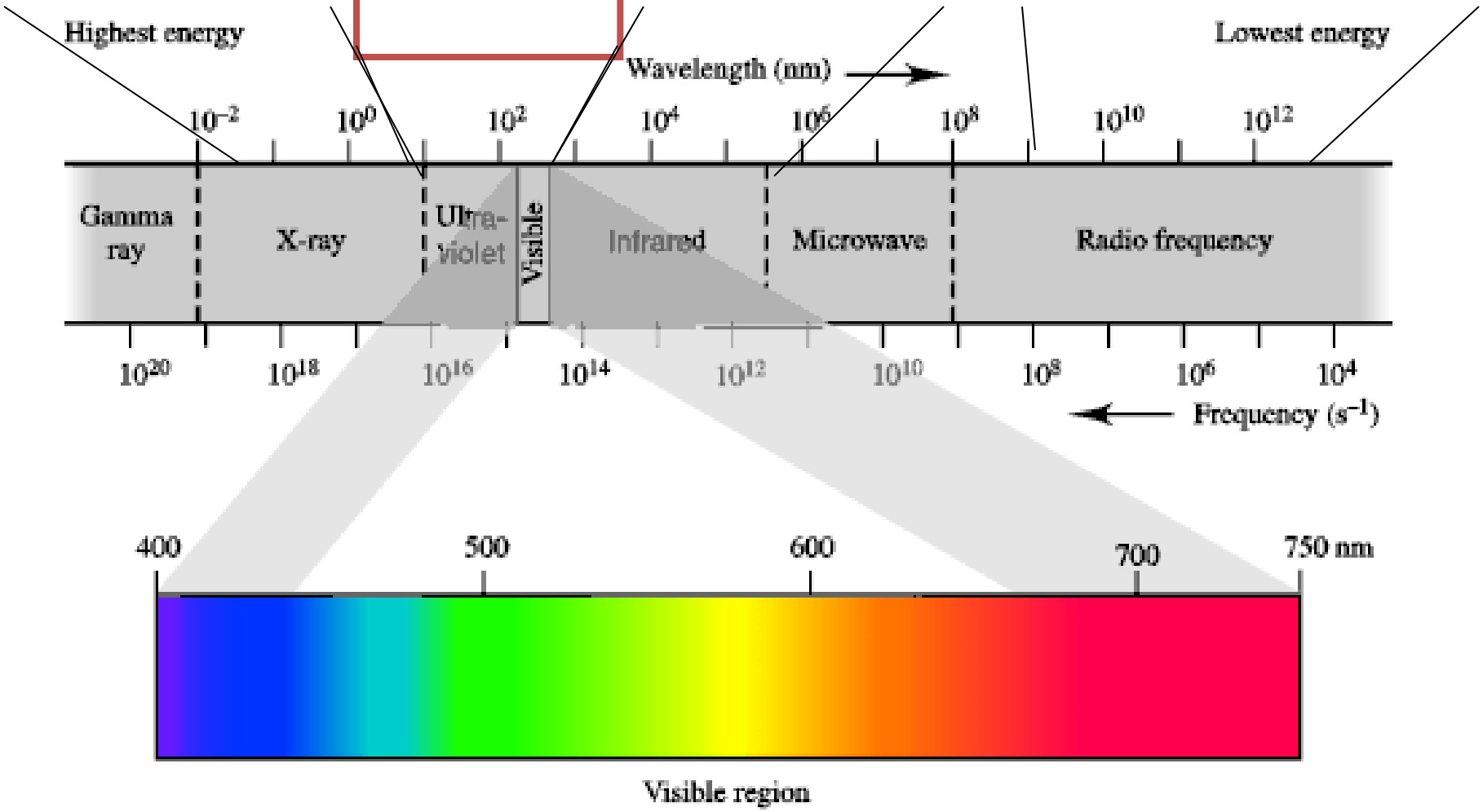
Electronic Excitation by UV/Vis Spectroscopy

X-ray:
core electron excitation

UV: valance electronic excitation

IR: molecular vibrations

Radio waves:
Nuclear spin states (in a magnetic field)



Spectroscopic Techniques and Common Uses

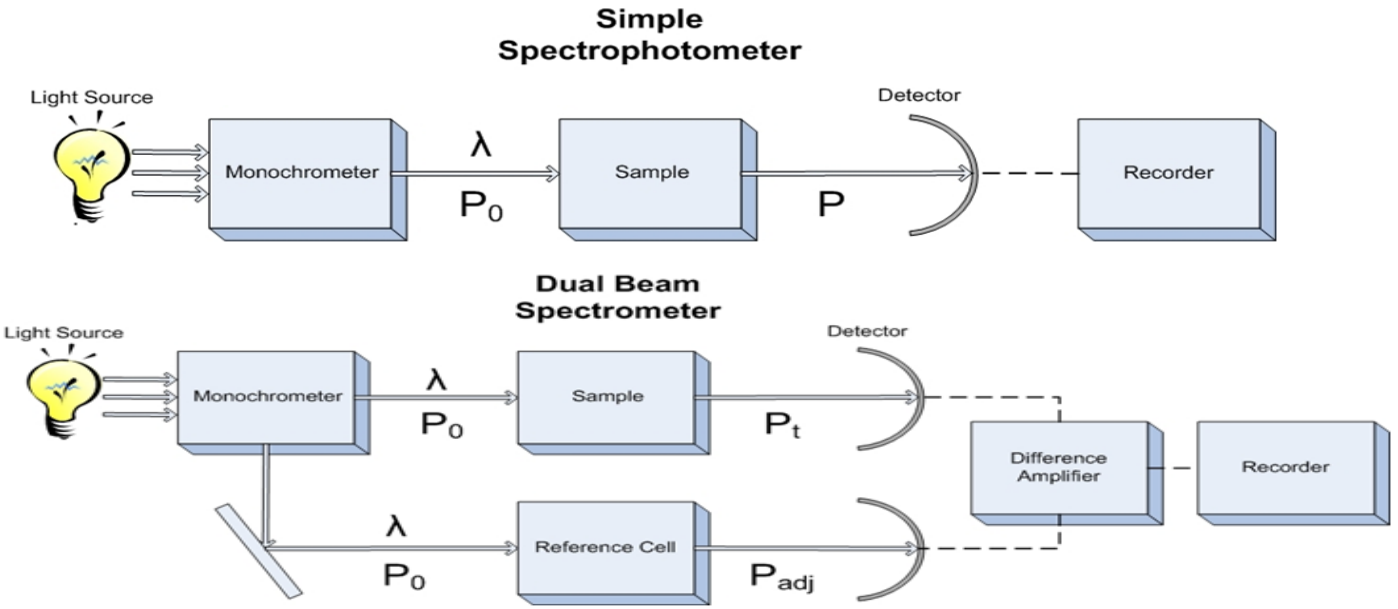
UV-vis	UV-vis region	Quantitative analysis/Beer's Law
Atomic Absorption	UV-vis region	Quantitative analysis Beer's Law
FT-IR	IR/Microwave	Functional Group Analysis
Raman	IR/UV	Functional Group Analysis/quant
FT-NMR	Radio waves	Structure determination
X-Ray Spectroscopy	X-rays	Elemental Analysis
X-ray Crystallography	X-rays	3-D structure Anaylysis

Different Spectroscopies

- UV-vis – electronic states of valence e/d-orbital transitions for solvated transition metals
- Fluorescence – emission of UV/vis by certain molecules
- FT-IR – vibrational transitions of molecules
- FT-NMR – nuclear spin transitions
- X-Ray Spectroscopy – electronic transitions of core electrons

The Types of Spectrophotometer

- Single beam spectrophotometer:
- Double beam spectrophotometer: the source light is split into two beams, one which travels through the sample, and another that is sent through a blank or standard solution. Both beams are then read by separate transducers and the difference between the two is recorded as the corrected transmittance.



Different modes of Spectrophotomety

- Quantitative mode: One Absorbance point in One wavelength
- Kinetic mode: Absorbance in one wavelength against Time.
- Spectrum mode: Absorbance against different wavelengths (Scan)
- Thermal Mode: Absorbance in one wavelength against Time (Sigmoid of Thermal denaturation of protein or DNA).

Quantitative Spectroscopy

- Beer's Law

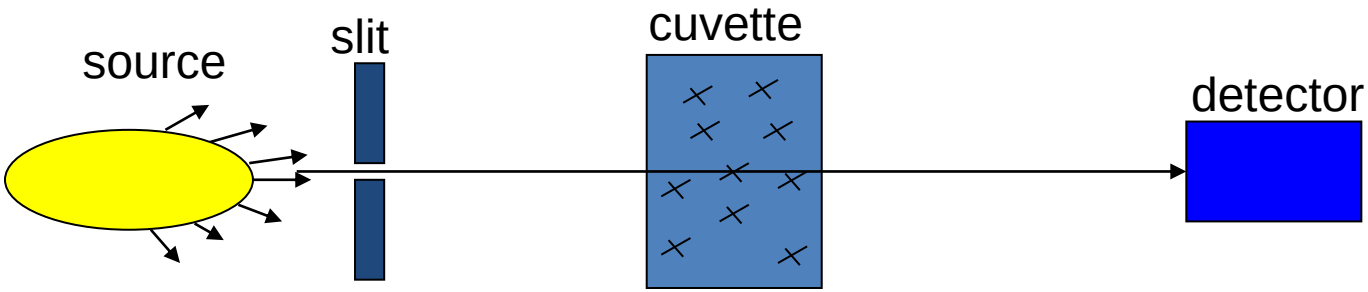
  $A = \xi bc$

  ξ is molar absorptivity (extinction coefficient)

  b is path length

  c concentration

Beer's Law



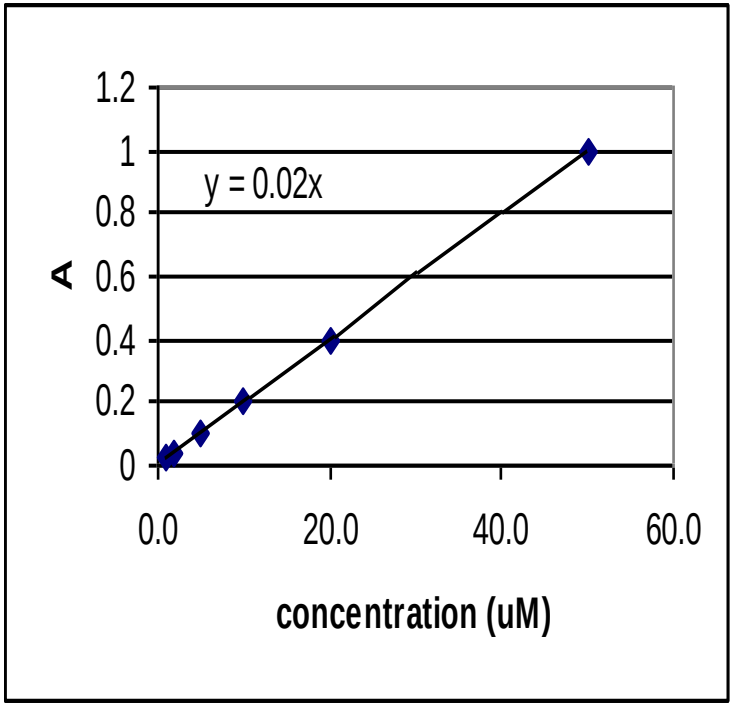
- $A = -\log T = \log(I_0/I) = \xi bc$
- $T = I_{solution}/I_{solvent} = I/I_0$
- Works for monochromatic light
- Compound x has a unique ξ at different wavelengths

Characteristics of Beer's Law Plots

- One wavelength
- Good plots have a range of absorbances from 0.010 to 1.000
- Absorbances over 1.000 are not that valid and should be avoided
- 2 orders of magnitude

Standard Practice

- Prepare standards of known concentration
- Measure absorbance at lmax
- Plot A vs. concentration
- Obtain slope
- Use slope (and intercept) to determine the concentration of the analyte in the unknown

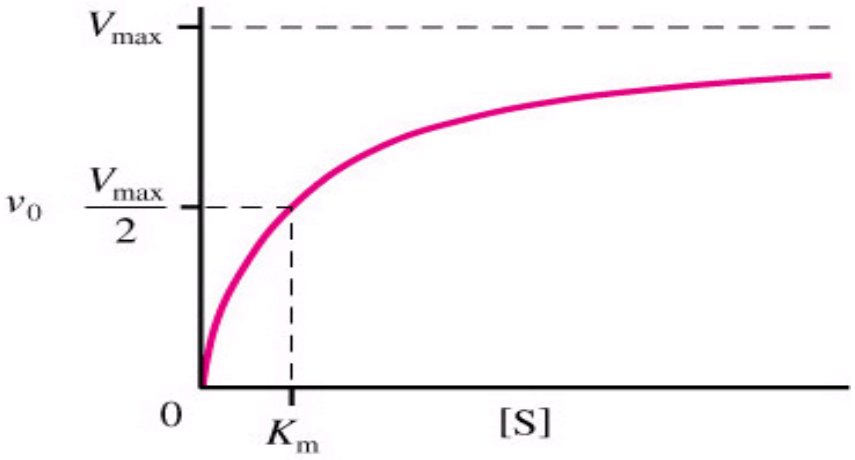


Kinetic Spectroscopy

- Absorbance vs Time
- Enzyme Kinetic

$$V = \frac{V_{max}[S]}{[S] + K_M}$$

- V_{max}
- K_m



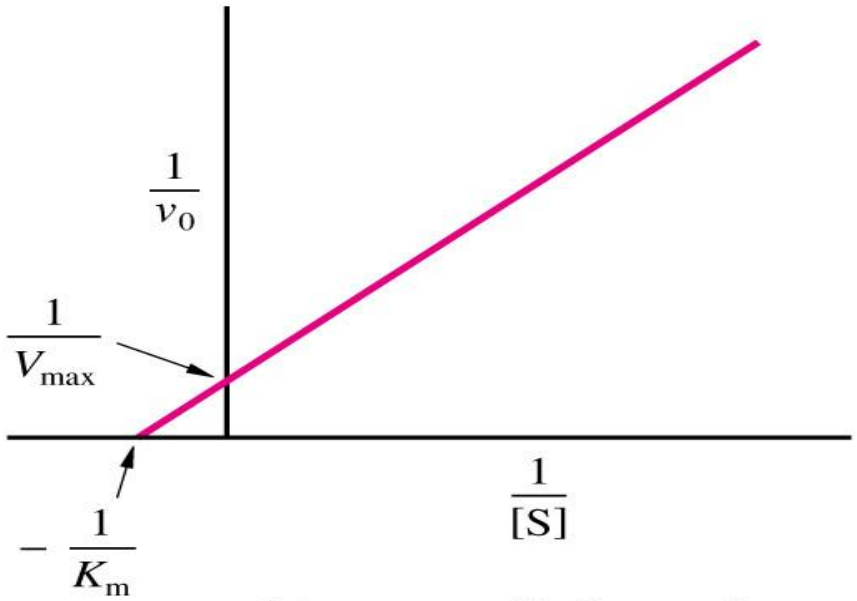
Lineweaver-Burk

- $v_0 = V_{max}[S]/(K_m+[S])$
- $1/v_0 = (K_m +[S])/(V_{max}[S])$
- $1/v_0 = (K_m /(V_{max}[S])) + [S]/(V_{max}[S]))$
- $1/v_0 = (K_m/V_{max})*1/[S] + 1/V_{max}$

Dean Burk

Hans Lineweaver

Lineweaver-Burke Plots



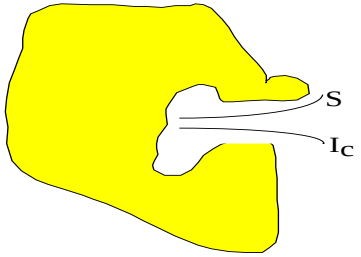
Lineweaver-Burk equation:

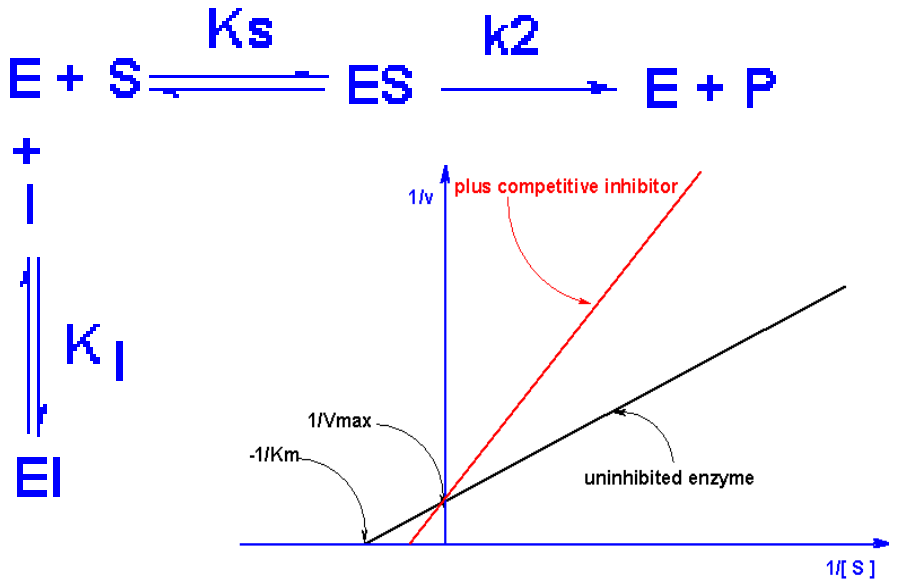
$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

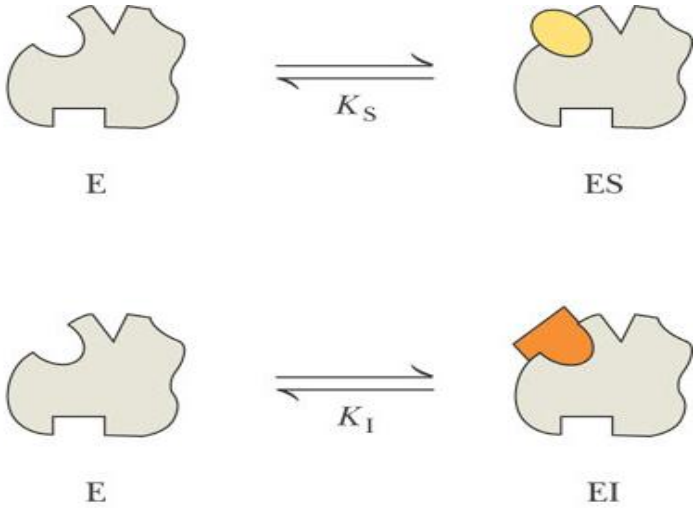
- L-B equation for straight line in Plot 1/[S] vs $1/V_o$
- X-intercept = $-1/K_m$
- Y-intercept = $1/V_{max}$

Lineweaver-Burke Plots-Enzyme Inhibition

- Competitive inhibition: Inhibitor binds at active site
- Prevents binding of substrate
- K_m goes up so -1/ K_m moves toward origin
- V_{max} unchanged so Y intercept unchanged

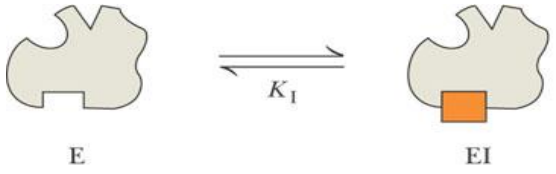


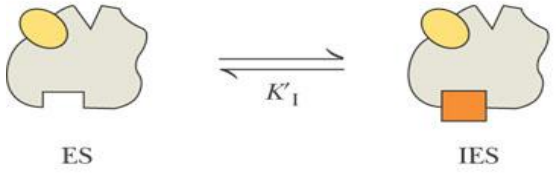


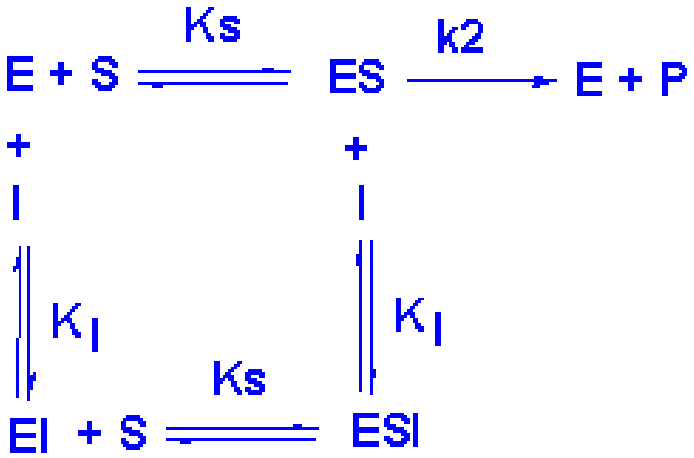


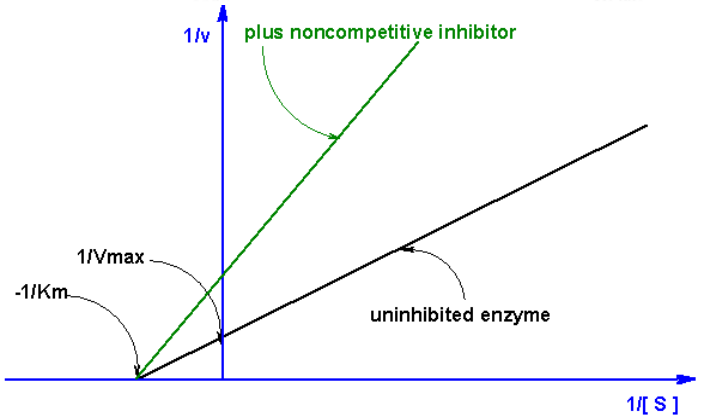
Lineweaver-Burke Plots-Enzyme Inhibition

- Non Competitive inhibition: Inhibitor binds distant from active site
- Interferes with turnover
- Decrease in $V_{max} \Rightarrow 1/V_{max}$ is larger
- X-intercept unaffected



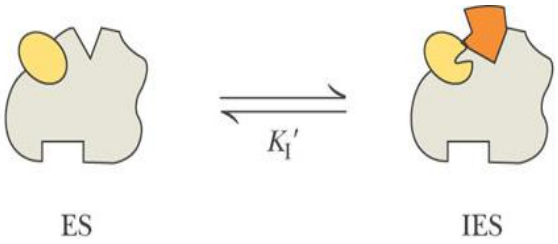


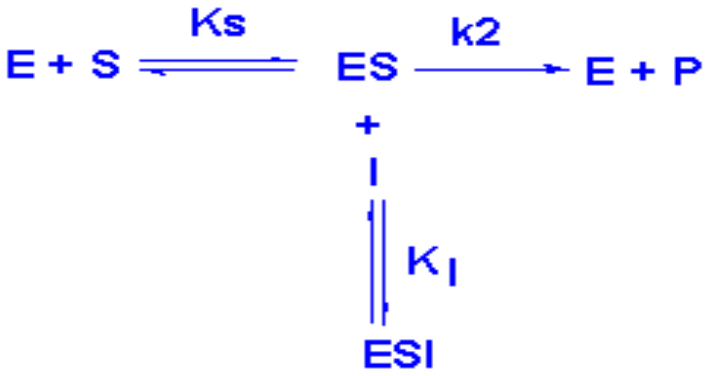


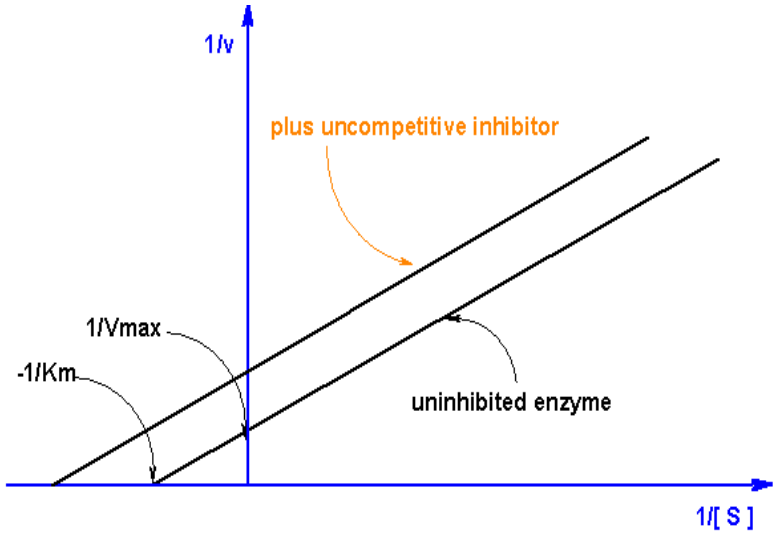


Lineweaver-Burke Plots-Enzyme Inhibition

- Un Competitive inhibition: Inhibitor binds to ES complex
- Removes ES, interferes with turnover
- K_m moves toward origin
- V_{max} moves away from the origin
- Slope (K_m/V_{max}) is unchanged

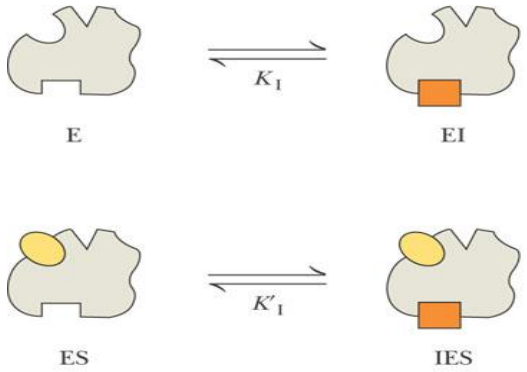


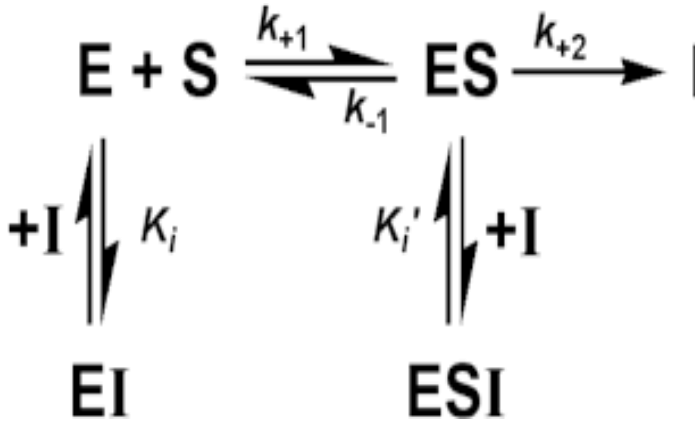


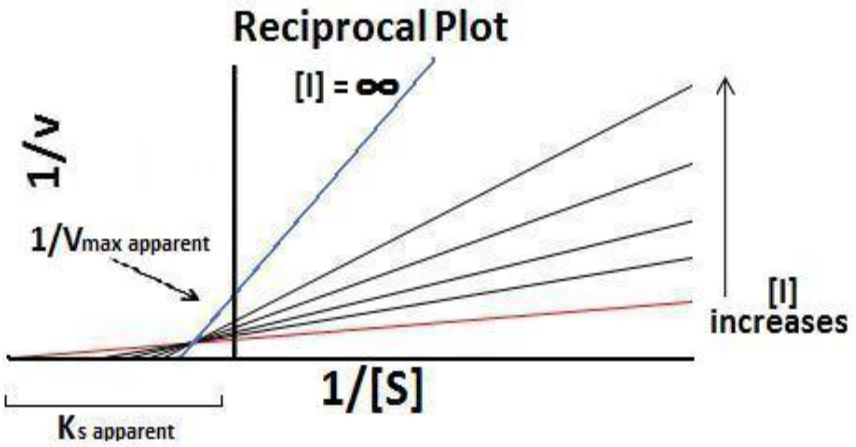


Lineweaver-Burke Plots-Enzyme Inhibition

- Mixed type of inhibition: Inhibitor binds distant from active site
- Inhibitor Interferes with Substrate
- Both the $V_{max} \Rightarrow 1/V_{max}$ and Km changed







The Important of Tyrosinase Inhibtion

Why Tyrosinase is important?

➢**Tyrosinase:** Widely distributed through the phylogenetic scale from lower to higher life forms

>In Insects: Sclerotization of exoskeleton

>>In mammals: Responsible for skin pigmentation

>>>In Fruits and vegetables: Undesired enzymatic browning

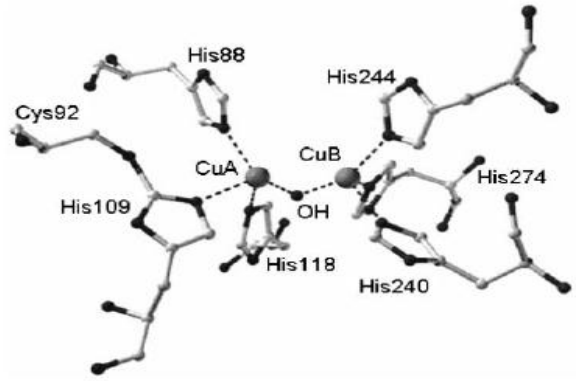
➢**Tyrosinase Inhibitors : They are natural and synthetic inhibitors**

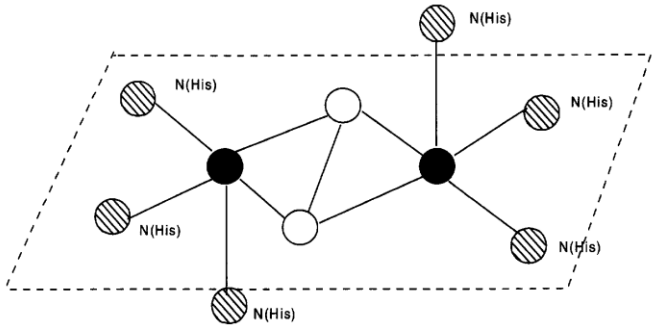
➢**Their inhibition constants (Ki) can be obtain by spectrophotometric techniques**

➢**Their kinetic of inhibition obeys from lineweaver burke plots.**

Active site structure

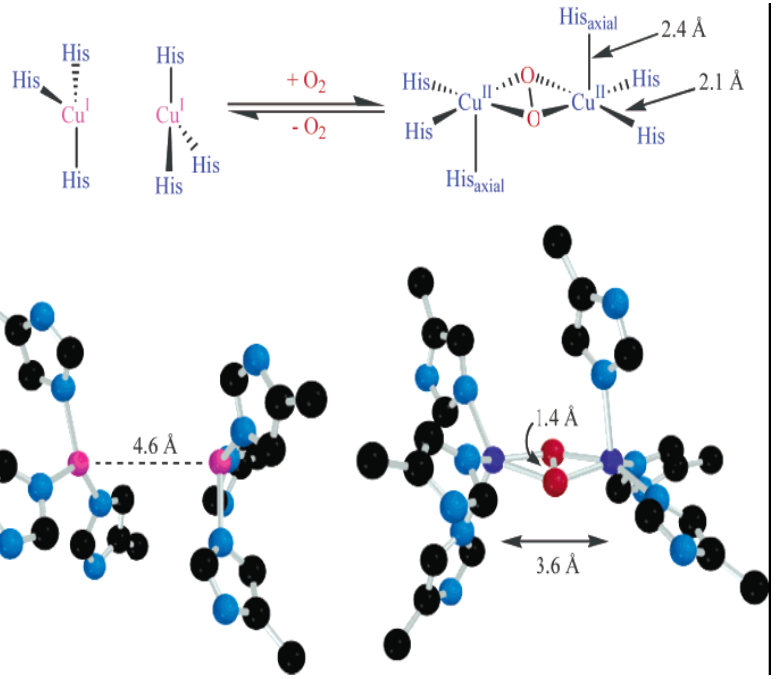
Met Form





Deoxy Form

Oxy Form

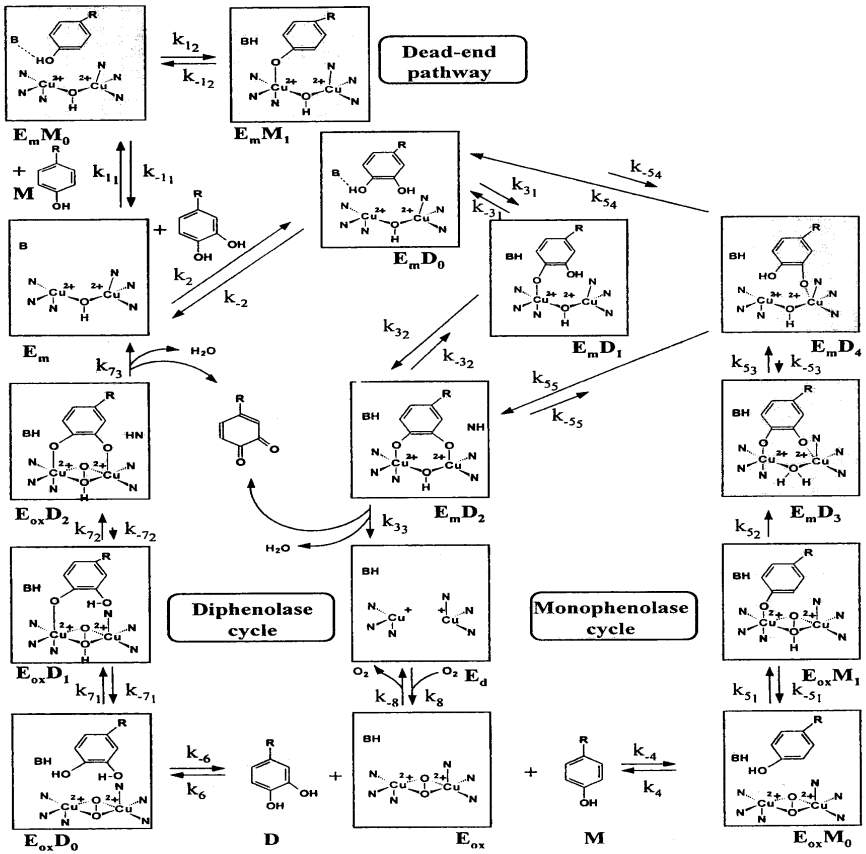


Mirica,L.M., Rudd, D. J., Vance, M. A., Solomon, E. I., Hodgson, K. O., Hedman, B., and Stack, T. D. P., J. AM. CHEM. SOC. 2006, 128, 2654-2665

➢Reaction Mechanism: Two distinct oxidation reaction

1: Oxidation of monophenols by oxygen (Cresolase)

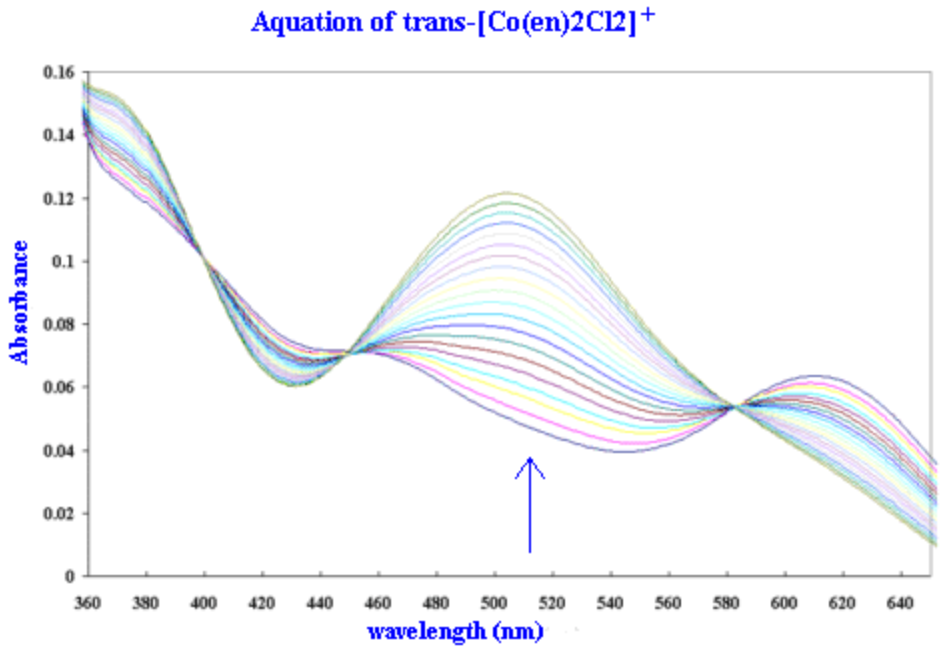
2: Oxidation of o-diphenols to o-quinones (Catecholase)



Olivares, C., Garcia-Boron, J.C. and Solano, F. (2002) Biochemistry 41, 679-686

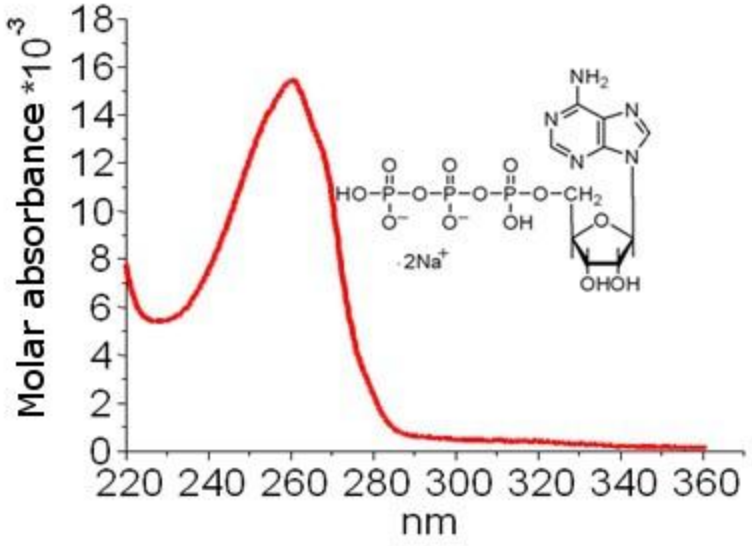
Spectrum other mode of Spectrophotometry

- Absorbance vs Wavelength
- Characterized the type of Biological compounds by verification of the maximum absorption wavelength (λ_{max})



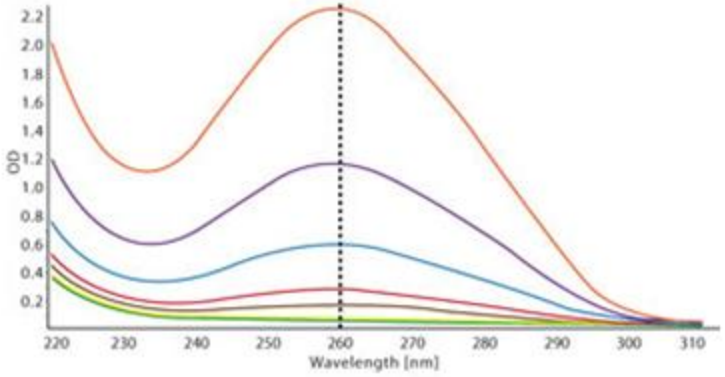
Some important Spectrums in Biology

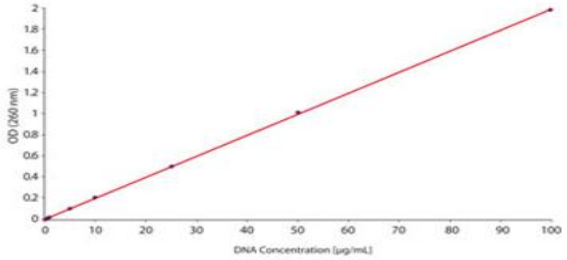
- **Absorption spectrum of ATP:**The absorption maximum of the molecule is at 260 nm due to its aromatic structure and having nucleotid.



Some important Spectrums in Biology

- DNA with the maximum absorption wavelength of 260nm



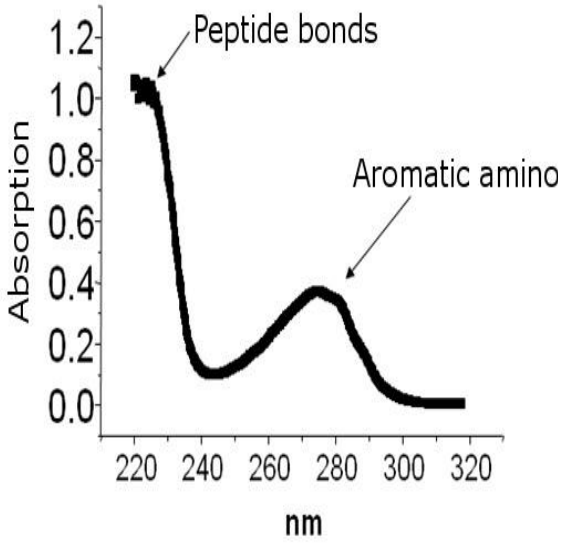


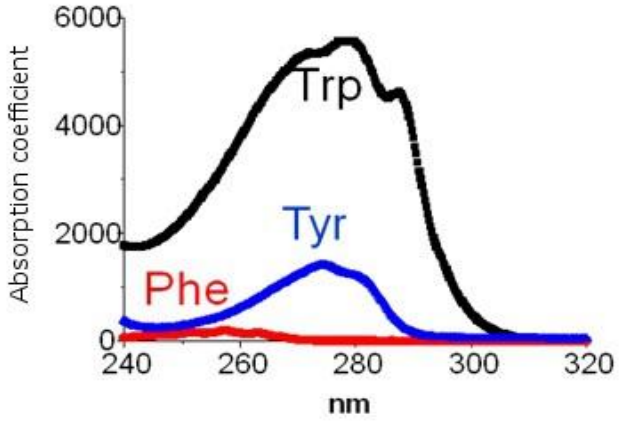
Extinction coefficients of different nucleic acids that can be used with absorbance 260 measurements and Beer's law.

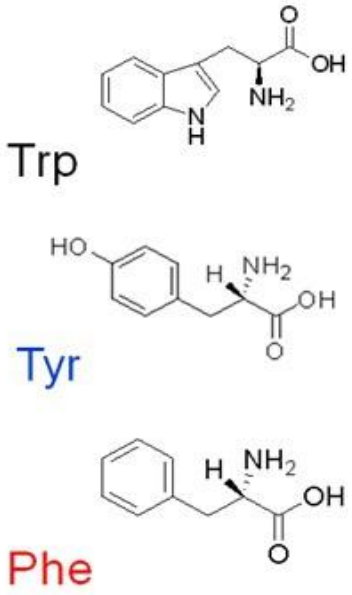
Nucleic acids	Extinction coefficient [$cm^{-1} \cdot M^{-1}$]	MARS Data Analysis Software
double stranded DNA	50	dsDNA template
single stranded DNA	33	ssDNA template
RNA	40	RNA template

Some important Spectrums in Biology

- Protein with the maximum absorption wavelengths in 230nm (peptide bonds), 280nm (Aromatic amino acids: Phe, Tyr, Trp) and the third pick related to the prosthetic group if it existence.

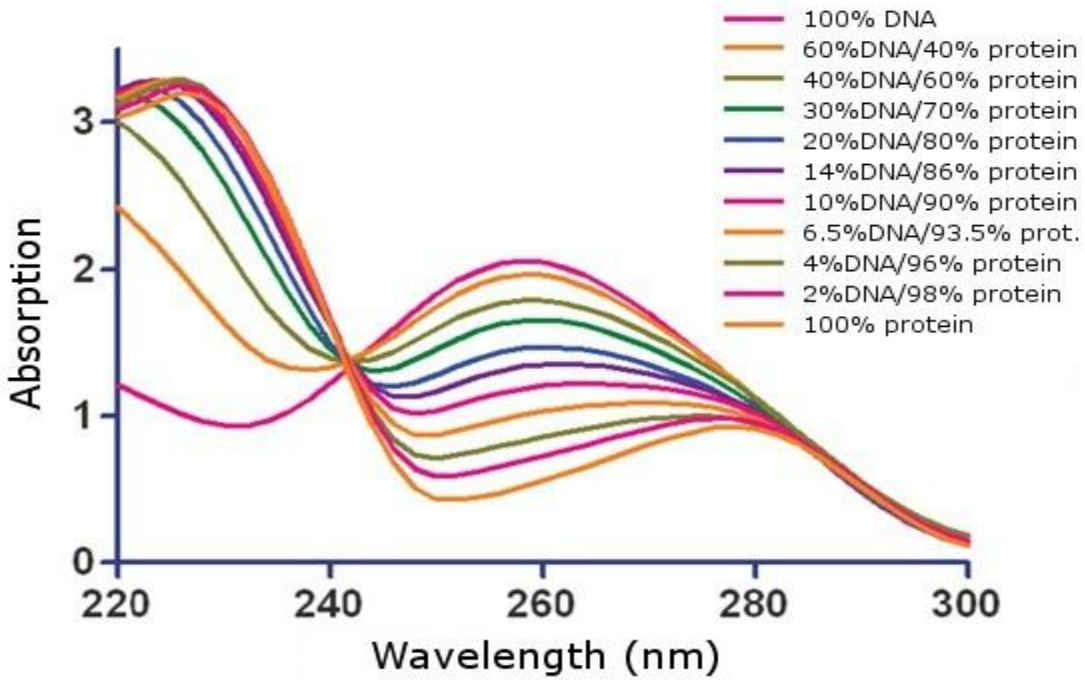






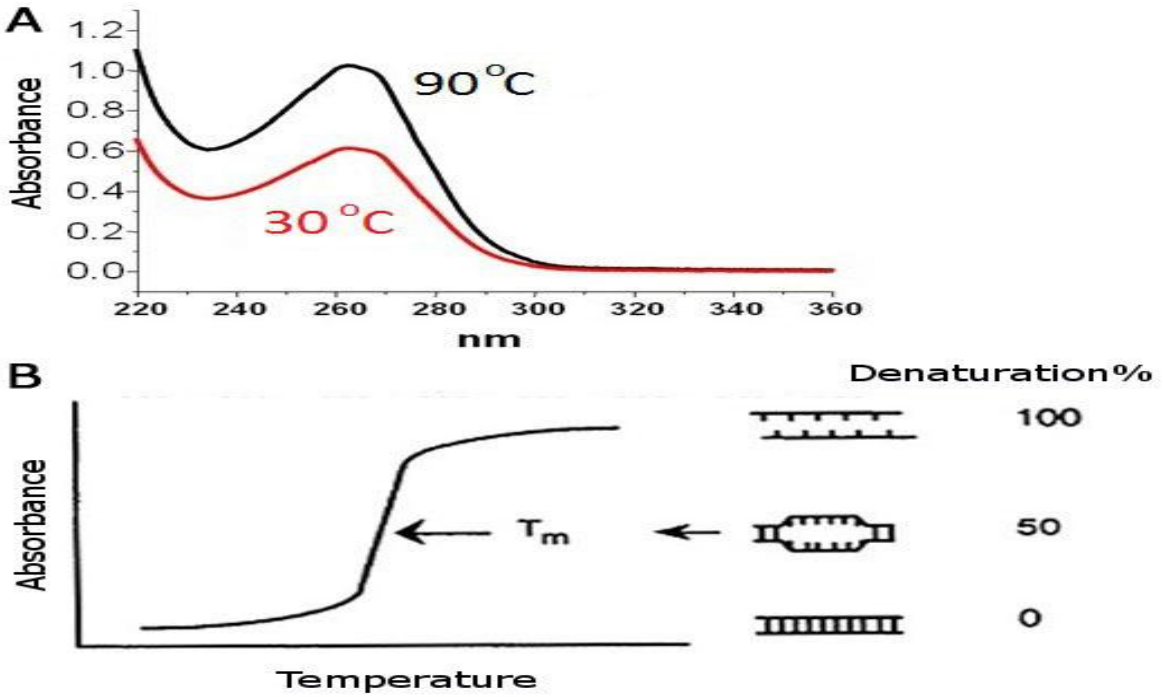
Determination of the purity of DNA and protein samples

- The ratio of DNA and protein in biological samples can be estimated from A_{260}/ A_{280} . In the case of a pure solution of DNA, A_{260}/ A_{280} = 1.8. The addition of protein to the solution will typically reduce this value.



Determination of thermodynamic parameters of proteins and DNA

- Hyperchromicity of DNA. A, Absorption spectra of a DNA molecule at 30°C (red) and 90°C (black). B, Temperature-induced change in absorbance at 260 nm, reflecting the denaturation (melting) of the double-stranded DNA structure.



- Scatchard Curves without cooperativity

Simple Ligand Binding

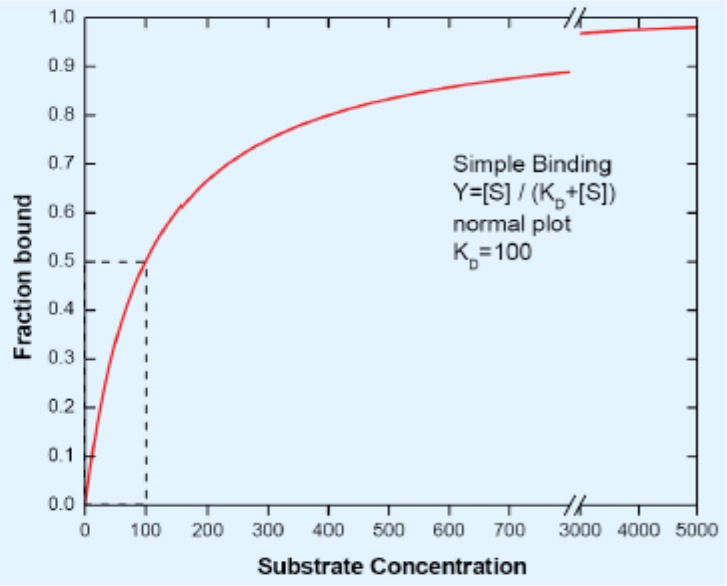
$$E + S \underset{K_D}{\rightleftharpoons} ES$$

$$\frac{[E][S]}{[ES]} = K_D$$

$$[E]_{total} = [E] + [ES]$$

$$Y = \frac{[ES]}{[E]_{total}} = \frac{[S]}{K_D + [S]}$$

Fraction of ligand bound



- Cooperativity in binding of ligand to protein: Hill equation

Highly cooperative ligand binding

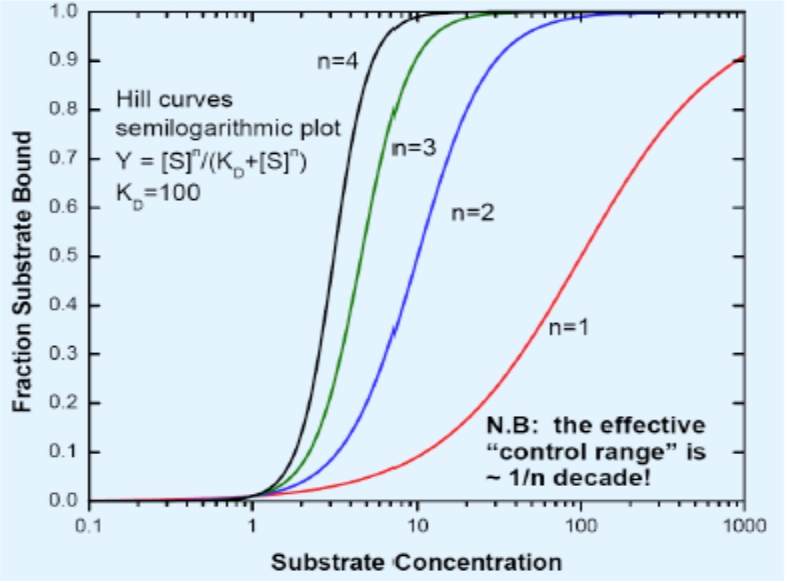
$$E + nS \rightleftharpoons ES^n \quad (K_D)$$

$$K_D = \frac{[E][S]^n}{[ES_n]}$$

$$Y = \frac{[ES_n]}{[E]_{total}} = \frac{[S]^n}{K_D + [S]^n}$$

$$\log \frac{Y}{1 - Y} = n \log[S] - \log K$$

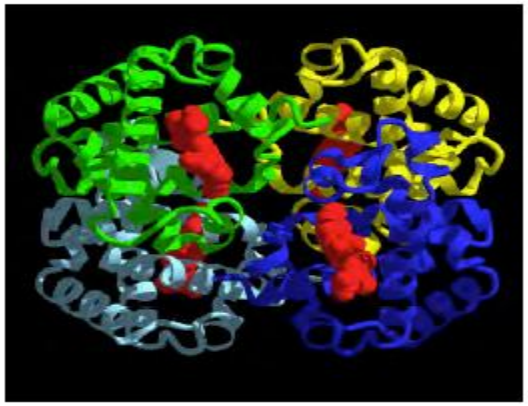
Hill coefficient

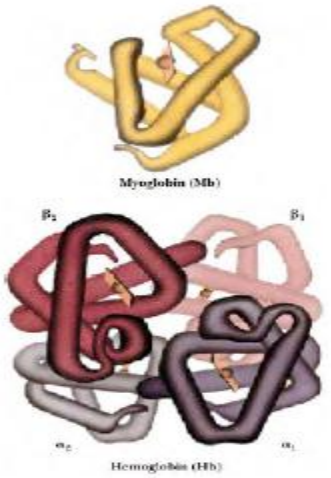


Ligand Binding

- Cooperativity in binding of ligand to protein: Hill equation

Hemoglobin >> Myoglobin × 4



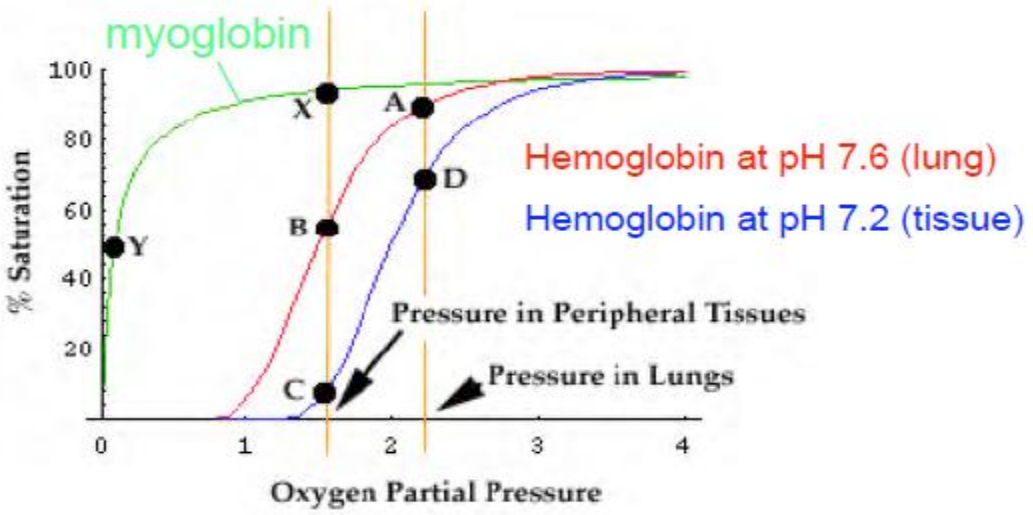


Max Perutz: hemoglobin
Nobel Prize in
Chemistry, 1962

John Kendrew: myoglobin
Nobel Prize in
Chemistry, 1962

Cooperative Oxygen Binding in Hemoglobin

- O_2 binding curve much steeper than myoglobin (cooperativity)
- shifts with pH (the Bohr effect)



Thank You